

09/432,906

-3-

B2  
C2

198:214 (1992), Small, J.V., *et al*, *J. Cell Sci.* 89:21 (1988), Lee, J.C., *et al*, *Biochem.* 19:6209 (1980).

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (page i).

In the Claims

Please cancel Claims 3, 6, 10, 18 and 27.

Please amend Claims 1, 4, 5, 8, 11, 15, 16, 17, 19, 20, 21, 22, 25, 28 and 31.

Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages ii - iv).

Sub  
3

1. (Amended) A method of cloning a mammal, comprising the steps of:
- combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
  - <sup>fusion</sup>impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned mammal; and
  - gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.

B4

4. (Amended) The method of Claim 2, wherein the somatic cell is an adult somatic cell or an embryonic somatic cell.
5. (Amended) The method of Claim 2, wherein the somatic cell is a fibroblast cell or an epithelial cell.

09/432,906

-4-

- 83 8. (Amended) A method of producing a transgenic mammal, comprising the steps of:
- a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;
  - b. <sup>fusion</sup>impregnating a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic mammal; and
  - c. gestating the embryo in step b, thereby causing the embryo to develop into the transgenic mammal.

- B 106 11. (Amended) A method of producing a mammalian nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo. <sup>and fusing</sup>

- B 1 15. (Amended) The method of Claim 11, wherein the oocyte is in metaphase II prior to activation to telophase II.

16. (Amended) The method of Claim 11, wherein the somatic donor cell is activated by reducing nutrients in the serum of the donor cell, and then exposing the donor cell to serum having an increased amount of nutrients.

- Sub 17. (Amended) The method of Claim 16, wherein the somatic activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G<sub>1</sub> phase, S phase, and G<sub>2</sub>/M phase.

- B 8 19. (Amended) The method of Claim 11, wherein combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte further includes fusing the activated donor cell with the activated oocyte.

09/432,906

-5-

20. (Amended) The method of Claim 11, wherein combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte further includes microinjecting the nucleus of the somatic activated donor cell into the activated, enucleated oocyte.

21. (Amended) A method of producing a protein of interest in a mammal, comprising the steps of:

- B8  
cancel
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo, wherein the nucleus from the somatic activated donor cell encodes the protein of interest;  
*fusion*
  - b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of a cloned mammal;
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal; and
  - d. purifying the protein of interest from the cloned mammal.

22. (Amended) The method of Claim 21, wherein purification of the protein of interest is expressed in tissue, cells or bodily secretion of the cloned mammal.

25. (Amended) A method of producing a heterologous protein in a transgenic mammal comprising the steps of:

- B9
- a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo, wherein the nucleus from the somatic activated donor cell encodes the heterologous protein;

09/432,906

-6-

- B9  
amended
- b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the nuclear transfer embryo into a cloned mammal;
  - c. gestating the embryo in step b., thereby causing the embryo to develop into a cloned mammal that expresses the heterologous protein; and
  - d. recovering the expressed heterologous protein from the cloned mammal.

- B10
28. (Amended) The method of Claim 25, wherein the genetically engineered nucleus includes an operatively linked promoter.

- B11
31. (Amended) A method of enucleating an oocyte having a meiotic spindle apparatus, comprising exposing the oocyte to at least one compound that destabilizes the meiotic spindle apparatus.

39. (New) A method of cloning a mammalian fetus, comprising the steps of:
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to <sup>fusion</sup> thereby form a nuclear transfer embryo;
  - b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned fetus.
40. (New) The method of Claim 39, wherein the activated donor cell is in a stage of a mitotic cell cycle selected from the group consisting of: G<sub>1</sub> phase, S phase, and G<sub>2</sub>/M phase.
41. (New) The method of Claim 39, wherein the somatic cell is an adult somatic cell or an embryonic somatic cell.

09/432,906

-7-

42. (New) The method of Claim 39, wherein the somatic cell is a fibroblast cell or an epithelial cell.
43. (New) The method of Claim 39, wherein the oocyte is enucleated chemically, by X-ray irradiation, by laser irradiation or by physical removal.
44. (New) A method of cloning a non-human mammal, comprising the steps of:
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
  - b. <sup>fusion</sup>impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the non-human cloned mammal; and
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the non-human cloned mammal.
45. (New) A method of producing a transgenic non-human mammal, comprising the steps of:
- a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;
  - b. <sup>fusion</sup>impregnating a non-human mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic non-human mammal; and
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic non-human mammal.
46. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated

B12  
SCG

09/432,906

-8-

oocyte in telophase II and of the same species as the donor cell, to thereby form a non-human nuclear transfer embryo.

- sub 50
- B12
47. (New) A method of cloning a non-human mammalian fetus, comprising the steps of:
- combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
  - <sup>fusion</sup>impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and
  - gestating the embryo in step b., thereby causing the embryo to develop into the cloned non-human mammalian fetus.
48. (New) A method of cloning a mammal, comprising the steps of:
- combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
  - <sup>fusion</sup>impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned mammal; and
  - gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.
49. (New) A method of producing a transgenic mammal, comprising the steps of:
- combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar

09/432,906

-9-

*Sub  
Set  
each*

- body and an extruding second polar body, and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;
- b. impregnating a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic mammal; and
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic mammal.

- B12*
50. (New) A method of producing a nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo.

*Sub  
Set*

- from*
51. (New) A method of cloning a mammalian fetus, comprising the steps of:
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
  - b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and
  - c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammalian fetus.

52. (New) A method of cloning a non-human animal, comprising the steps of:
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;

09/432,906

-10-

- b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the non-human cloned mammal; and
- c. gestating the embryo in step b., thereby causing the embryo to develop into the non-human cloned mammal.
53. (New) A method of producing a transgenic non-human animal, comprising the steps of:
- a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;
- b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic non-human mammal; and
- c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic non-human mammal.
54. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a non-human nuclear transfer embryo.
55. (New) A method of cloning a non-human fetus, comprising the steps of:
- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte in telophase II and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
- b. impregnating a non-human mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned fetus; and



09/432,906

-11-

- c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned non-human mammalian fetus.

56. (New) A method of cloning an animal, comprising the steps of:

- a. combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a nuclear transfer embryo;
- b. impregnating a mammal of the same species as the nuclear transfer embryo with the nuclear transfer embryo under conditions suitable for gestation of the cloned mammal; and
- c. gestating the embryo in step b., thereby causing the embryo to develop into the cloned mammal.

57. (New) A method of producing a transgenic animal, comprising the steps of:

- a. combining a genetically engineered nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar body, and of the same species as the donor cell, to thereby form a transgenic nuclear transfer embryo;
- b. impregnating a mammal of the same species as the nuclear transfer embryo with the transgenic nuclear transfer embryo under conditions suitable for gestation of the transgenic mammal; and
- c. gestating the embryo in step b., thereby causing the embryo to develop into the transgenic mammal.

58. (New) A method of producing a non-human nuclear transfer embryo, comprising combining a nucleus from a somatic activated donor cell with an activated, enucleated oocyte derived from an oocyte having a first polar body and an extruding second polar